



## Impact of Pb-Zn mining activity on surficial sediments of Lake Kalimanci (FYR Macedonia)

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**Abstract:** The activities of the Sasa mine have led to industrial discharge being released into Lake Kalimanci since the beginning of mining activity in 1954. For this reason, an ecological risk assessment is urgently required. In the present study, surficial lake sediments were analyzed using inductively coupled plasma mass spectrometry (ICP-MS), with all geochemistry data processed using Statistica 8. In order to assess the environmental pollution status, 2 environmental factors were established, while for the determination of chemical fractionation of toxic metals a sequential extraction procedure was carried out. Concentrations of As, Mo, Zn, Ni, Co, Pb, Cd, and Cu in surficial Lake Kalimanci sediments were in the ranges of 58.6–77.2, 2.30–4.60, 7056–20,900, 47.4–79.3, 18.8–25.3, 4863–16,300, 46.8–136, and 328–1162 mg/kg, respectively. Based on values of the enrichment factor and geoaccumulation index, the surficial sediment of Lake Kalimanci is strongly polluted in terms of most of the examined metals. The results of the sequential extraction procedure revealed the majority of the investigated toxic metals (Zn, Pb, Ni, Cu, and Cd) to be strongly bonded to the exchangeable fraction and the rest (As and Mo) to the oxidizable fraction. The geochemistry, environmental factor values, and toxic metal extraction characteristics of Lake Kalimanci sediments represent a serious environmental risk for an extensive area.

**Key words:** Pollution, tailings dam failure, toxic metal contamination, sequential extraction procedure, surficial lake sediments, Lake Kalimanci, Republic of Macedonia

### 1. Introduction

Across the world, many researchers have carried out investigations into anthropogenic contamination of entire ecosystems by focusing on the role of toxic metals (Issa *et al.* 1996; Szymanowska *et al.* 1999; Ebrahimpour & Mushrifah 2008; Singh *et al.* 2008; López *et al.* 2010; Yan *et al.* 2010; Gómez-Álvarez *et al.* 2011). In this regard, toxic metals are amongst the most widespread of the various pollutants that originate from industrial activities, particularly from mining and smelting waste sites (e.g., Salomons 1995; Hochella *et al.* 2005). Through ore extraction and processing, large amounts of waste material (containing toxic metals) are produced and stored, much of it highly toxic. Environmental concerns in such mining areas are primarily related to the physical, chemical, and biological disturbances of the surrounding landscape and unstable mine tailings, dust emissions, and acid mine drainage (AMD), which may be transported into rivers.

Both active and abandoned mine sites have produced vast quantities of inactive sulfide-rich mine wastes in various regions of the world, including Macedonia. The make-up and concentration of potentially toxic elements varies extensively with different deposit types, but can

include As, Pb, Cd, Fe, Cu, Zn, and, in some cases, Tl, Se, Mo, and Re. It is well known that under highly acidic conditions, metal ions including those of Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn become more soluble and mobile and are released from tailings in levels toxic to plants (Kerstner & Förstner 1995; Villalobos-Castañeda *et al.* 2010).

In aquatic systems that are affected by smelting and/or mining activities, metals may be present as dissolved ions and complexes, in suspension, as colloidal ions, or in solid form within sediments and in biological materials. The concentration of these metal ions strongly depends on local biological processes, redox potential, ionic strength, pH, the activity of organic and inorganic chelators, and scavenging processes (Arjonilla *et al.* 1994). When local hydrological conditions change, toxic metals in the sediment can be released into the water, thereby deteriorating its quality and affecting aquatic organisms (Adham *et al.* 1999; Rashed 2001; Pagnanelli *et al.* 2004; Zhong *et al.* 2006).

Toxic metals entering the fluvial network can be transported along hydrological gradients for hundreds of kilometers in relatively short times. The release of toxic metals can occur both in dissolved and particulate form.

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When released in particulate form or when adsorbed to particles, toxic metals can settle and be deposited under favorable hydraulic conditions in river bottom sediments, channels, and flood plains, as well as in natural and artificial lakes (Audry *et al.* 2004).

Toxic metals in the surficial sediments of natural and artificial lakes or water-storage facilities are mostly associated with fine-grained particles. Contaminated lake sediments are generally regarded as an important component of the overall lake system, since they provide a bank of environmental information regarding the extent of both natural and anthropogenic pollution (Dauvalter & Rognerud 2001; Belzile *et al.* 2004; Das 2005; Bibi *et al.* 2007). The mobility and bioavailability of toxic metals and other elements stored in lake bottom sediments depend on many different factors, such as temperature, pH, pressure, salinity, the chemical form of the element, and redox conditions (Jernström *et al.* 2010). However, due to their rapid sedimentation rates, the sediments of water reservoirs are often considered to be little affected by early-stage diagenetic processes (Callender 2000) and thus provide a well-preserved and well-documented history of toxic metal input derived from, for instance, a catastrophic spillage of acid mine tailings into the adjacent environment.

The total concentration of metals in sediments is generally not sufficient to assess environmental impact and thus an estimation of the bioavailable fraction is necessary. The bioavailable fraction is defined as the amount of metal that is bound to wastes (easily exchangeable ions, metal carbonates, oxides, sulfides, organometallic compounds, etc.) and that can be exchanged with organisms and incorporated into their structures (Vangronsveld & Cunningham 1998; Weisz *et al.* 2000; Yu *et al.* 2001). In this sense, sequential extraction therefore permits us to precisely identify the origins of the metals and to better understand their geochemical cycles and mobility. It can be inferred from geochemical partitioning studies what amount of toxic metal could be more biologically available (Luoma 1983). The most commonly used technique to determine the different forms of metals present in environmental samples is sequential extraction. Numerous sequential extraction methods are typically used, differing in terms of the reagent type, experimental conditions, and number of steps required (e.g., Tessier *et al.* 1979; Gibson & Farmer 1986; Kerstern & Förstner 1991; Oughton *et al.* 1992; Quevauviller *et al.* 1997; Gomez-Ariza *et al.* 2000; Wenzel *et al.* 2001; Quevauviller 2002). The most commonly isolated phases of sequential extraction schemes are exchangeable, carbonate-bound, Fe-Mn oxide-bound (reducible), organic (oxidizable), and residual (Anju & Banerjee 2011).

The current study is the first to investigate not only the concentrations of As, Mo, Zn, Ni, Co, Pb, Cd, and

Cu present in the surficial sediment of the artificial mine that has impacted Lake Kalimanci in eastern Macedonia, but also the binding forms in which they occur using sequential extraction. The results are combined in order to more accurately define the enrichment of toxic metals derived from the massive acid mine tailings spill that took place in 2003. The environmental risk associated with total toxic metal concentrations and their geochemical fractionation in sediment will also be evaluated.

## 2. Materials and methods

### 2.1. The study area

Situated in eastern Macedonia, artificial Lake Kalimanci lies in the vicinity of the small town of Makedonska Kamenica, about 5 km from the Sasa-Toranica ore district (Figure 1). With a length of 14 km and width of 0.3 km, the lake covers an area of 4.23 km<sup>2</sup> and contains approximately  $127 \times 10^6$  m<sup>3</sup> of water. The basic purpose of Lake Kalimanci is the storage of water that is used to irrigate around 30,000 ha of mainly rice fields in the Kočani Valley (around the city of Kočani) and Ovce Pole, which extends all the way from Lake Kalimanci towards the city of Delčevo and the border with Bulgaria. As a result, the lake is characterized by large changes in water level, with dry season levels occasionally decreasing to only a few centimeters in the inner (deeper) areas. Such changes mean that surficial lake sediments are often exposed to oxic conditions. In winter and spring, the maximum depth of the lake can reach 80 m, with the exception of the northern margin of the lake where the Kamenica River enters (Figure 1). This area is very shallow and swampy and, as a consequence, contains the largest amount of Sasa tailings dam material. The lake has 2 outflow channels; the western is 36 km long and is used to irrigate fields in the Kočani Valley, while the eastern is 98 km long and carries water to Ovce Pole. Lake Kalimanci also has 2 inflowing tributaries that feed the lake and drain the southern Osogovo Mountains. The first of these tributaries is the Bregalnica River, which, together with its tributaries, drains igneous metamorphic and sedimentary rock of Precambrian to Holocene age, as well as mine waste and tailings from both abandoned and active Pb-Zn mines, and polymetallic mineralized materials from the Serbo-Macedonian Massif. The second and most severely polluted tributary is the Kamenica River, which drains mine waste including tailings, mill sewage, and mine effluent derived from the Sasa Pb-Zn polymetallic ore deposit. The Kamenica River then flows directly into Lake Kalimanci (Figure 1).

### 2.2. Geology and environmental setting of the study area

The Sasa Pb-Zn deposit lies within the Sasa-Toranica mining district in the Osogovo Mountains (eastern Macedonia), which has been in production since 1954. The geology of the Toranica-Sasa ore field comprises various rocks of both metamorphic and igneous origin, with the latter of



**Figure 1.** Geographical location map of Lake Kalimanci.

Tertiary age. The most abundant lithologies in the area are believed to be closely associated with mineralization. The Sasa deposit consists of peneconcordant tabular ore bodies that wedge out following a transition to nonmineralized metasomatites and lens-sheeted ore bodies. The most economically valuable mineralization is closely related to the quartz-graphite schists, with the ore consisting mainly of galena, sphalerite, chalcopyrite, and pyrite. Other studies have revealed more details as to the complexity of the deposit's mineralogy, including the presence of galena, sphalerite, chalcopyrite, pyrite, pyrrhotite, magnetite, martite, bornite, enargite, tetrahedrite, marcasite, barite, native gold, cubanite, and native bismuth (Serafimovski & Aleksandrov 1995; Stojanov *et al.* 1995; Serafimovski *et al.* 2006).

Mining in the region is carried out following the conventional underground method. The Sasa tailings reservoir is man-made, constructed with remnant material from ore processing and located between 2 hills in the Osogovo Mountains, approximately 10 km north of the small town of Makedonska Kamenica. Mine tailings produced from the milling process are discharged into a tailings pond as slurry. Both tailings and milling material are weakly cemented and locally exposed to weathering,

thus promoting the production of AMD and posing a severe threat in terms of trace metal pollution to both the immediate area and those further downstream (towards the Kamenica River and Lake Kalimanci). Running through the Sasa mine, the Kamenica River receives waste discharge from the mine before finally flowing into Lake Kalimanci.

In 2003, a major environmental disaster took place in eastern Macedonia, with part of the Sasa mine tailings dam collapsing and causing an intensive flow of tailings through the Kamenica valley. Between 70,000 and 100,000 m<sup>3</sup> of tailings material was discharged into Lake Kalimanci, causing significant ecological damage.

### 2.3. Sampling methods

Seven surface sediment samples were collected from Lake Kalimanci in September 2008 (Figure 2), with sample pH values ranging from 5.5 to 7.5. A pH of 5.5 was measured only at 1 sampling location (III-3), where an increased pyrite content was observed. At all the other sampling locations, pH ranged between 7.2 and 7.5. Sampling was carried out during a dry period (no precipitation – summer season) when the lake water levels were lower and the sediments were exposed to oxidation processes. The lake water was also low due to water release from annual maintenance.

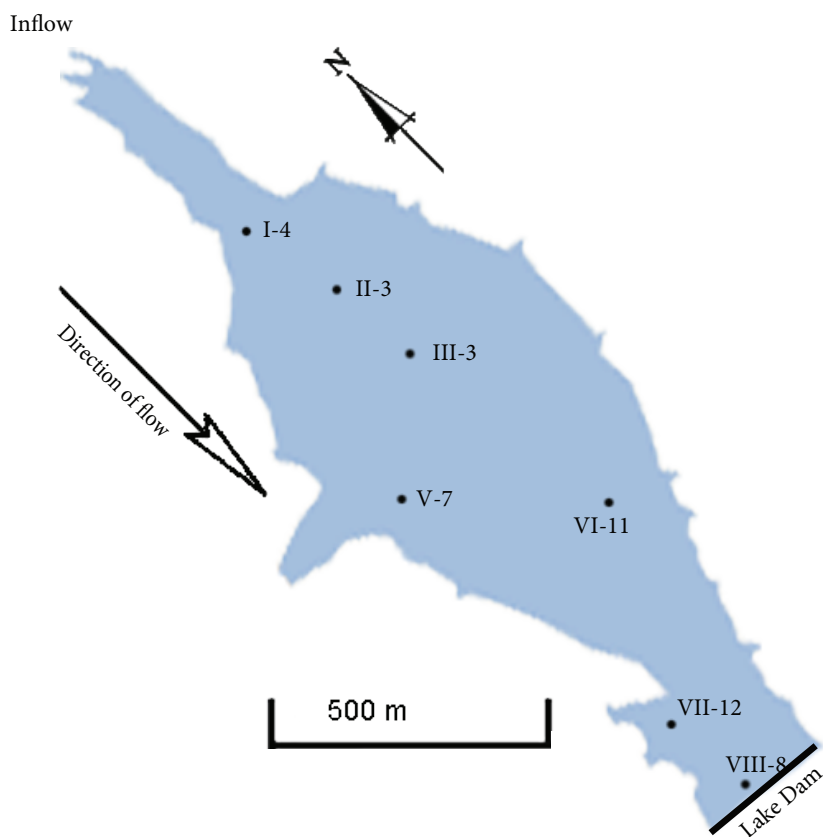


Figure 2. Distribution of the sampling stations across Lake Kalimanci.

Therefore, the surficial lake sediments were exposed to oxidation processes for 1 day before the sampling began. Samples were collected using a plastic spade, transferred to precleaned plastic bags, and then stored in the laboratory at 4 °C. After collection, the samples were oven-dried before undergoing dry sieving at a temperature of 50 °C for 48 h until a constant weight was attained. After being sieved through a 0.315-mm polyethylene sieve to remove plant debris, samples were finally homogenized to a fine powder in a mechanical agate grinder for subsequent analysis.

The water quality of Lake Kalimanci and the Kamenica River has not been analyzed yet, but will be a matter of further investigation when the dissolved and suspended load of the lake, the inflow and outflow of the rivers, and the seasonal effects and variations of chemistry with discharge are studied.

#### 2.4. Total geochemistry and sequential extraction

The chemical analyses were obtained from the commercial ACME Laboratories in Vancouver, Canada. Determination of abundances required 0.5 g of sampled materials, which were leached in hot (95 °C) aqua regia and afterwards analyzed using inductively coupled plasma mass spectrometry (ICP-MS). According to the official laboratory reports, the accuracy and precision of

the surface sediment analyses were assessed via the use of international reference materials provided by ACME such as USGS standards STD SO-18, STD CSC, STD DS7, STD OREAS76A, and STD R3A as well as repeated measurements of 5 of our samples. The repeated analyses of different sample aliquots indicated a relative standard deviation of  $\pm 5\%$ .

The binding forms of heavy metals in the surficial sediment samples were established according to the sequential chemical extraction method (Tessier *et al.* 1979; Hall & Pelchat 1999, Cappuyns *et al.* 2006). The trace metal content of different chemical fractions of the samples was determined via various extractions of lake sediment components. While such schemes are widely used in environmental geochemistry, a sequential extraction method devised by ACME laboratories was applied in order to estimate pollutant speciation and bioavailability in the sediment samples. The most commonly isolated phases in such sequential extraction schemes include a water-soluble fraction (leaching stage 1), an exchangeable + carbonate fraction (leaching stage 2), an oxidizable-organic fraction (leaching stage 3), an Fe-Mn oxide-bound-reducible fraction (leaching stage 4), and a residual + reducible fraction (leaching stage 5).



The surficial sediment samples, each weighing 1 g, were placed in screw-top test tubes. To each sample was added 10 mL of leaching solution, the caps were screwed on, and the tubes were subjected to the appropriate extraction procedure depending on the stage of the leach. For sequential leaching, the sample was leached, centrifuged, decanted, and washed, and then the residue was leached again in a 5-step process from the weakest to the strongest solution or chemical reagent (Table 1). The concentration of heavy metals in the solution was then measured using a PerkinElmer 6000 ICP-MS system for the determination of 60 or more elements. QA/QC protocol incorporated a sample duplicate to monitor the analytical precision, a reagent blank to measure background, and an aliquot of in-house reference material to monitor accuracy.

The mobility and bioavailability of toxic metals in lake sediments both depend on their binding form; therefore, each sample was operationally defined as follows:

**Water-soluble fraction** (leaching stage 1 – F1): Metals were extracted and rolled for 2 h before undergoing ICP-MS analysis. In this fraction, metals are relatively labile and thus may be more readily leachable and potentially bioavailable in the environment (Hsu & Lo 2001). Van Herck and Vandecasteele (2001) reported that this fraction consists of metals that are easily soluble (chlorides, sulfates), highly mobile, and potentially bioavailable, and thus the leaching of metals from this fraction is a major environmental concern (Filgueiras *et al.* 2002).

**Exchangeable fraction** (leaching stage 2 – F2): This fraction includes metals that are adsorbed onto the solid surface by relatively weak electrostatic interactive forces (weakly bound toxic metal species) and which can then be released through ion-exchange processes, as well as metals that are precipitated with carbonates (Filgueiras *et al.* 2002). Adsorption-desorption reactions or a lowering of pH cause changes in ionic composition and could consequently cause the remobilization of metals from this fraction. Metals in this fraction are the most readily available for biota and thereby very labile. It is also the fraction with the most labile bond to the sediment and, therefore, the most dangerous for the environment.

**Oxidizable fraction** (leaching stage 3 – F3): This fraction corresponds to elements that occur as oxidizable

minerals and also to organically bound metals. Metals bound to this fraction can be released under oxidizing conditions (Filgueiras *et al.* 2002; Fuentes *et al.* 2004). This fraction represents the amount of metal bound to organic matter and sulfides, which can also be released under oxidizing conditions. Such conditions can occur, for example, if sediments are resuspended (by dredging, currents, flooding, tides, etc.) and the sediment particles come into contact with oxygen-rich water (Morillo *et al.* 2002).

**Reducible fraction** (leaching stage 4 – F4): This fraction usually consists of oxides of Mn and Fe that are extracted together and that act as a cement, being present as nodules between particles or coating them. Elements are strongly bound to these oxides, but are thermodynamically unstable in anoxic conditions (Filgueiras *et al.* 2002; Fuentes *et al.* 2004). However, this phase also consists of metals bound to iron and manganese oxides that may be released if pH and/or redox conditions of the sediment change, which may be caused, for example, by the activity of microorganisms (Morillo *et al.* 2002).

**Reducible + residual fraction** (leaching stage 5 – F5): This fraction corresponds to elements linked to amorphous Fe hydroxides (reducible part) that are expected to be released to the environment under reducing conditions. Metals in this fraction are retained within the crystal lattice of minerals and inside crystallized oxides. They can only be mobilized as a result of weathering and therefore have only very long-term effects (Filgueiras *et al.* 2002; Kazi *et al.* 2002). The metals that correspond primarily to this fraction are those that are associated with minerals in which they form part of the crystalline structure and, as a result, are unlikely to be released from sediments. Thus, elements in the residual phase are considered to be stable and inert, and they are not susceptible to remobilization except under very harsh conditions and over very long time scales (Ikem *et al.* 2003).

## 2.4. Statistics

The obtained results were analyzed using linear regression and analysis of covariance (ANCOVA) with Statistica 8. Regression calculations were done with 95% confidence intervals.

**Table 1.** Sequential extraction procedure – fractions and chemical reagents.

Step	Fraction	Reagents/1 g sample
1	Water-soluble	20 mL distilled water
2	Exchangeable and carbonate bound	20 mL ammonium acetate
3	Organic (oxidizable)	20 mL 0.1 M sodium pyrophosphate ( $\text{Na}_3\text{P}_2\text{O}_7$ )
4	Reducible	60 °C, 20 mL 0.1 M hydroxylamine hydrochloride
5	Reducible + residual	90 °C, 20 mL 0.25 M hydroxylamine hydrochloride

## 2.5. Normalization and environmental factors

Assessing the anthropogenic metal enrichment of surficial Lake Kalimanci sediments is problematic. Some studies have used worldwide mean values (e.g., Turekian & Wedepohl 1961; Birch & Taylor 2000) as an estimate of preindustrial metal concentrations, whereas others have chosen to actively sample presumed preindustrial sediments to obtain background metal values (e.g., Swennen *et al.* 1998). The use of a global mean in the examination of regional or local anthropogenic metal contamination has limitations (Matschullat *et al.* 2000).

In order to differentiate between the lithogenic and anthropogenic origin of toxic metals in Lake Kalimanci sediments, toxic metal concentrations were normalized to Sc. As a conservative (very insoluble) element hosted in a variety of ferromagnesian minerals (Fronzl 1970), Sc was selected instead of other conservative elements such as Ti or Zr due to its wide distribution in crustal rocks. This is different from Ti and Zr, which are mainly concentrated in ilmenite and zircon, respectively (Steinmann & Shotyk 1997).

### 2.5.1. Enrichment factor

In order to evaluate the extent of the toxic metal pollution affecting Lake Kalimanci sediments, an enrichment factor (EF) was calculated as follows:

$$EF = (Me/Sc)_{sed} / (Me/Sc)_{sed\ Dojran}$$

where  $(Me/Sc)_{sed}$  is the concentration ratio of a given metal to Sc in the Kalimanci sediment sample, and  $(Me/Sc)_{sed\ Dojran}$  is the average ratio of the same metal to Sc in the reference (nonpolluted) Lake Dojran in Macedonia.

Sediments from Lake Dojran were sampled in 1998 with plastic corers, and 6 samples were taken. The analytical procedure was the same as described in Section 2.4. The measured values for Sc were a minimum of 6.40 mg/kg, a maximum of 25.8 mg/kg, and an average of 16.8 mg/kg (Table 2). The sediments from Lake Dojran were chosen as reference material because this area belongs to the Serbo-Macedonian Massif like the Osogovo Mountains, where Lake Kalimanci is located. No mining activity is carried out in the vicinity of Lake Dojran.

Enrichment factor values can be interpreted as representing the level of toxic metal pollution as suggested by Birth (2003), with the assessment criteria based on the EF values presented in Table 3.

### 2.5.2. Geoaccumulation index ( $I_{geo}$ )

A second criterion with which to evaluate the intensity of historical toxic metal pollution of Lake Kalimanci sediments is the geoaccumulation index devised by Förstner and Müller (1973) and Müller (1979):

$$I_{geo} = \log_2((M)_{sed} / 1.5 (M)_{sed\ Dojran}) ,$$

where  $(M)_{sed}$  is the concentration of a given toxic metal and  $(M)_{sed\ Dojran}$  is the mean concentration of the same metal in the sediments of the reference site (Lake Dojran).  $I_{geo}$  is associated with a qualitative scale of pollution intensity, as shown in Table 4.

## 3. Results and discussion

### 3.1. Total toxic metal concentration and comparison of individual toxic metal concentrations with natural background values and other lakes

The present study focuses on changes in the concentration and chemical fractionation of metals in Lake Kalimanci sediments, with the aim of identifying the dispersal of toxic metals by riverine transport from the Sasa mine. At Lake Kalimanci, most mining waste material is retained behind a dam (Figure 2). Seven sediment samples (I-4 to VIII-8) were obtained from the lake along a transect extending from the village of Makedonska Kamenica to the lake outlet at the Lake Kalimanci dam (Figure 2). As can be seen from Table 5, the concentrations of toxic elements decreased towards the lake dam (Figure 2). The characteristic sediment metal enrichment is evident in the upper part of the lake (samples II-3, III-3, and V-7; Table 5) at the inflow of the Kamenica River. The highest amounts of all metals except Co were measured at location II-3 in the northern part of Lake Kalimanci, with As at 77.2 mg/kg, Mo at 4.6 mg/kg, Zn at 20,900 mg/kg, Ni at 79.3 mg/kg, Pb at 16,300 mg/kg, Cd at 136 mg/kg, and Cu at 1162 mg/kg.

### 3.2. Statistics

Table 6 shows the maximum, minimum, and mean concentrations of Zn, Mo, Cu, Pb, Ni, As, Cd, and Co in the surficial sediments of Lake Kalimanci in eastern Macedonia.

With respect to mean upper continental crust levels (Wedepohl 1995), the mean metal content values at Lake Kalimanci were more than 800, 450, 170, 50, and 25 times

**Table 2.** Minimum, maximum, and average content of selected metals in sediments from Lake Dojran.

	As	Mo	Zn	Ni	Co	Pb	Cd	Cu	Sc
Min.	3.00	0.50	46.0	27.0	9.00	16.0	0.50	18.0	6.40
Max.	57.0	3.00	109	75.0	30.0	56.0	4.00	62.0	25.8
Avg.	39.4	1.63	101	61.7	20.5	34.3	2.40	45.6	16.8

**Table 3.** Enrichment factor (EF) value in relation to pollution intensity as suggested by Birth (2003).

EF < 1	No enrichment
1 < EF < 3	Minor enrichment
3 < EF < 5	Moderate enrichment
5 < EF < 10	Moderately severe enrichment
10 < EF < 25	Severe enrichment
25 < EF < 50	Very severe enrichment
EF > 50	Extremely severe enrichment

higher for Cd, Pb, Zn, As, and Cu, respectively, and more than twice as high for Ni, Co, and Mo. The average toxic metal concentrations in the surficial sediments of Lake Dojran were lower than those of Lake Kalimanci, with each ranging as follows (mg/kg): Zn 46.0–109, Mo 0.50–3.00, As 30.0–57.0, Ni 27.0–75.0, Co 9.00–30.0, Pb 16.0–56.0, Cd 0.50–4.00, and Cu 18.0–62.0 (Table 2).

A Pearson correlation matrix was used to assess elemental associations and metal origins (Table 6). The results of the correlation matrix reveal a significantly positive correlation between Zn, Mo, As, Ni, Pb, Cd, and Cu, suggesting co-contamination from similar sources, in this case presumably the Sasa tailings dam. However, no correlations were noted between Zn and Co, which indicates that the Co content of the lake sediment is derived from a different source and/or has a natural origin or a different depositional nature.

### 3.3. Intensity of toxic metal pollution

#### 3.3.1. Enrichment factor

The calculation of EF values revealed that all studied toxic metals were enriched in the surficial Lake Kalimanci sediments. The highest average EF values were observed for Cd (800), Pb (445), and Zn (169) (Table 7), representing extremely severe enrichment according to the Birth scale (2003). The average EF value for As of 43.3 signifies very severe enrichment, while that for Cu (24.7) shows severe enrichment. The average EF values of Mo, Ni, and Co (2.09, 2.71, and 2.16, respectively) represent minor enrichment in the Lake Kalimanci surficial sediments.

In summary, the toxic metal contamination of Lake Kalimanci sediments varies between metals, with the following ranking from highest to lowest: Cd > Pb > Zn > As > Cu > Ni > Co > Mo. According to these calculated EF values, the study area is seriously polluted due to the release of large amounts of metals derived from mining activities.

#### 3.3.2. Geoaccumulation index

The calculated geoaccumulation index values for the surface sediments from Lake Kalimanci are presented in Table 8. According to Müller's scale (1979) the results

**Table 4.** Geoaccumulation index ( $I_{geo}$ ) in relation to pollution intensity, after Müller (1979).

$I_{geo}$	Pollution intensity
>5	Very strongly polluted
4–5	Strongly to very strongly polluted
3–4	Strongly polluted
2–3	Moderately to strongly polluted
1–2	Moderately polluted
0–1	Unpolluted to moderately polluted
<0	Unpolluted

(average  $I_{geo}$  values) reveal the surficial Lake Kalimanci sediments to be very strongly polluted with Pb (7.48) and Zn (6.39), strongly to very strongly polluted with Cd (4.66), strongly polluted with Cu (3.23), unpolluted to moderately polluted with Mo (0.57) and As (0.19), and unpolluted with Co (–0.47) and Ni (–0.63). This pollution has likely existed since the major environmental disaster of 2003.

In terms of  $I_{geo}$  classification, the intensity of lake sediment toxic metal pollution follows the relative order of Pb > Zn > Cd > Cu > Mo > As > Co > Ni. More importantly, Lake Kalimanci sediments contain levels of Cd, Zn, Pb, and Cu that could be severely detrimental to aquatic biota. Sediment resuspension during floods or due to anthropogenic activities (e.g., dredging) can remobilize reduced particulate toxic metals that may then be oxidized and thus pose a significant risk for biota (Audry *et al.* 2004).

### 3.4. Sequential extraction data

The environmental hazard represented by the contamination of the Lake Kalimanci surficial sediments not only depends on the total metal content, but also on its mobility and bioavailability, as determined by sequential extraction. The extracted percentage values of As, Mo, Zn, Ni, Co, Pb, Cd, and Cu found in the 7 surficial lake sediment samples after each sequential extraction step and the percentage of the sum of all fractions are shown in Figure 3.

**Arsenic:** A major quantity of As was found to be associated with the oxidizable fraction (F3) at all 7 locations (7.29–14.4 mg/kg), with the highest proportion (60.31%) of As in the oxidizable fraction observed in the sediment sample from VI-12. The least mobile was the As in the water-soluble fraction (F1), with less than 0.20 mg/kg found at II-3 and levels below 0.5% at all other locations. The mobility of As in the other fractions was as follows: 2.17–5.39 mg/kg in the reducible + residual fraction (F5), 1.52–3.30 mg/kg in the reducible fraction (F4), and 0.72–2.66 mg/kg in the exchangeable fraction (F2).

**Molybdenum:** A significant amount of Mo was available as part of the oxidizable fraction (F3), with the highest levels found at location II-3 (0.49 mg/kg) and the

**Table 5.** Concentrations in mg/kg of toxic metals in the investigated samples.

Element concentration (mg/kg)	As	Mo	Zn	Ni	Co	Pb	Cd	Cu
Location								
I-4	68.8	2.90	10,700	47.4	18.8	9357	74.0	672
II-3	77.2	4.60	20,900	79.3	24.3	16,300	136	1162
III-3	73.2	3.70	14,000	50.7	19.7	10,900	89.6	723
V-7	66.0	3.70	12,600	53.7	20.5	9472	81.1	596
VI-11	58.6	2.50	11,600	53.2	24.3	7557	77.6	546
VII-12	66.4	2.70	9326	56.1	25.3	5144	53.5	398
VIII-8	61.5	2.30	7056	48.5	23.5	4863	46.8	328

lowest at VII-12 (0.10 mg/kg). The next most-populated phase was the reducible + residual fraction (F5), with values of Mo ranging from 0.04 mg/kg to 0.09 mg/kg. Mo was the most mobile of all the studied elements in the water-soluble fraction (F1), with observed values ranging from 0.02 mg/kg to 0.11 mg/kg at location II-3. This was followed by the exchangeable fraction (F2) (range: 0.01–0.10 mg/kg), and finally the least mobile Mo was found in the reducible fraction (F4), with values ranging from 0.01 mg/kg to 0.02 mg/kg. Mo was likely detected within the water-soluble fraction (F1) due to the low ionic strength or pH of Lake Kalimanci.

**Zinc:** In contrast to As and Mo, a significant amount of Zn, ranging from 1648 to 4446 mg/kg at locations VII-12 and II-3, was found in the exchangeable fraction (F2), and the least was found in water-soluble form (F1), with values of 5.44–19.4 mg/kg at the same locations as that of the exchangeable fraction. Zn was also highly mobile in the reducible fraction (F4), with levels varying from 949 mg/kg at location VII-12 to 1773 mg/kg at location II-3. In the investigated lake sediments, Zn was weakly associated with the oxidizable fraction (F3) and the reducible + residual fraction (F5).

**Nickel:** A significant fraction of Ni in the sediment samples from all 7 sampling locations was present in the reducible fraction (F4) and exchangeable fraction (F2). The lowest and highest proportions of Ni (7.36–19.3 mg/kg) in the reducible phase were detected at locations VII-12 and II-3, respectively, with samples from the same locations also containing the lowest and highest levels of Ni (6.43 and 23.8 mg/kg, respectively) in the exchangeable fraction.

**Cobalt (Co):** The predominant chemical form of Co was found in the reducible fraction (F4), with levels ranging from 3.87 mg/kg (I-4) to 8.32 mg/kg (VIII-8). Co was thus the most mobile of all studied metals on the southern side of Lake Kalimanci (location VII-8). After the reducible fraction, Co occurred mainly within the exchangeable fraction (F2) at levels varying from 2.56 mg/kg to 5.57 mg/kg.

**Lead:** A large proportion of Pb was bound to the exchangeable fraction (F2), with levels varying from 1384 mg/kg to 6457 mg/kg. The highest amounts of extracted Pb were found on the northern side of Lake Kalimanci. Concentrations of Pb in the reducible phase (F4) were found to be the greatest along the middle of the lake, at levels ranging between 1004–2022 mg/kg.

**Table 6.** Basic statistics and Pearson correlation matrix showing interelemental relationships (N = 7).

	Mean	Min	Max	Std. dev.	Zn	Mo	As	Ni	Co	Pb	Cd	Cu
Zn	12,312	7056	20,900	4403	1.00							
Mo	3.20	2.30	4.60	0.83	0.93*	1.00						
As	67.4	58.6	77.2	6.41	0.78*	0.86*	1.00					
Ni	55.6	47.4	79.3	10.9	0.85*	0.74	0.61	1.00				
Co	22.3	18.8	25.30	2.60	0.02	−0.18	−0.25	0.47	1.00			
Pb	9085	4863	16,300	3900	0.97*	0.93*	0.82*	0.75	−0.20	1.00		
Cd	79.8	46.80	136	29.1	0.99*	0.90*	0.75	0.82*	−0.04	0.98*	1.00	
Cu	632	328	1162	273	0.97*	0.89*	0.82*	0.79*	−0.12	0.99*	0.98*	1.00

\*: Significant at  $P < 0.05$ .



**Table 7.** Enrichment factor values of 8 elements in samples from 7 sites in Lake Kalimanci.

Location	As	Mo	Zn	Ni	Co	Pb	Cd	Cu
I-4	29.6	1.25	97	1.53	1.21	302	487	17.4
II-3	57.1	3.40	326	4.40	2.69	904	1539	51.5
III-3	51.0	2.58	206	2.65	2.06	569	955	30.2
V-7	44.6	2.50	180	2.72	2.08	480	838	24.1
VI-11	39.6	1.69	157	2.70	2.46	383	803	22.1
VII-12	42.3	1.72	125	2.68	2.42	246	521	15.1
VIII-8	39.0	1.46	94	2.30	2.23	231	454	12.5
Average	43.3	2.09	169	2.71	2.16	445	800	24.7

**Cadmium:** Like Zn and Pb, the most mobile form of Cd was also found in the exchangeable fraction (F2), with the largest proportions detected in sediment samples from sites V-7 (39.7 mg/kg) and VI-11 (39.5 mg/kg) in the central part of Lake Kalimanci. Samples from these 2 locations also contained the highest relative levels of Cd found in the reducible phase (F4), at 4.92 mg/kg at site V-7 and 5.65 mg/kg at site VI-11.

**Copper:** In all the sediment samples, Cu was found to be strongly associated with the exchangeable phase (F2), closely followed by the oxidizable phase (F3). Samples containing the most Cu in the exchangeable phase were those from II-3 (107 mg/kg) and I-4 (102 mg/kg). Levels of Cu associated with the oxidizable fraction varied from 27.4 mg/kg (VII-12) to 90.3 mg/kg (II-3).

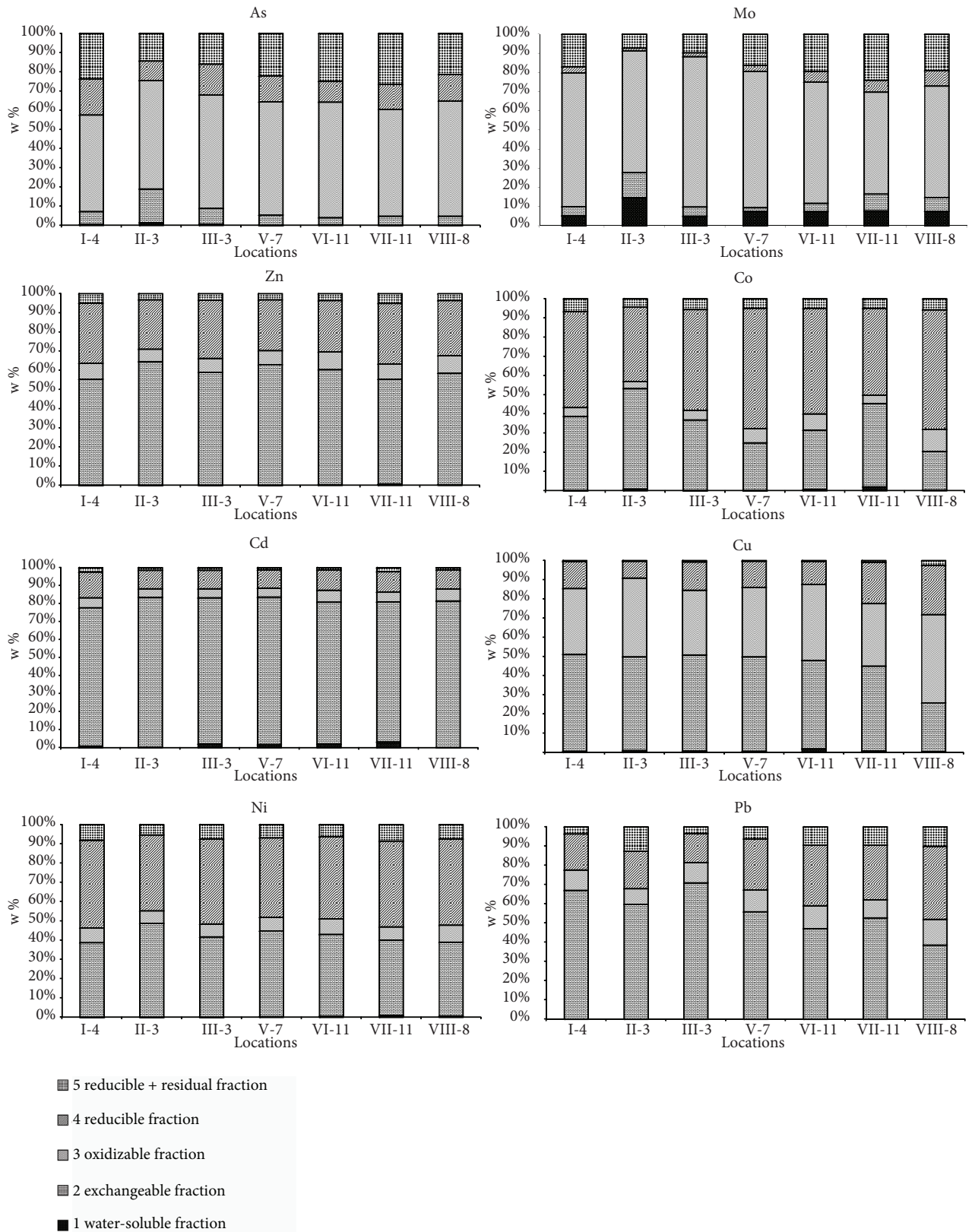
The results obtained from the sequential extraction give important information about the mobility of toxic metals in sediments (Li *et al.* 2001). The influence of toxic metals on the environment depends to a large extent on whether they occur in forms that can be taken up by fauna and flora.

In general (F1 + F2), the mobility of toxic metals in Lake Kalimanci sediments decreased in the order of

$Cd > Zn > Pb > Cu > Ni > Co > Mo > As$ . Cd, Pb, Zn, Ni, Co, and Cu were found to be highly mobile in the exchangeable fraction (F2), while Mo and As were the most exchangeable and highly mobile in the oxidizable fraction (F3) in all surficial sediment samples. The high association of elements (As, Mo, Cu) with the oxidizable fraction (F3) confirms the hypothesis of elemental fractionation being strongly affected by oxidizing conditions associated with low water levels during the dry season. Similar results were found in the fine-grained sediments of the Maharlu Saline Lake studied by Forghani *et al.* (2009), with the oxidizable fraction again being well represented. The majority of samples contained nearly no detectable Cu, Cd, Zn, Co, Pb, or Ni in the residual phase (F5), with As and Mo the only 2 metals detected to any significant degree. In any case, residual forms are not expected to be released under normal conditions in nature (Dean 2002). Therefore, they are typically considered to be inert or stable phases that cannot be mobilized and, as such, contribute to background values of sediment elemental content (Tessier *et al.* 1979). The reducible phase (F4) is the major metal host in estuarine sediments (Calamano *et al.* 1993). Ni and Co were highly bound to the reducible

**Table 8.**  $I_{geo}$  values in the surface sediments of Lake Kalimanci.

Location	As	Mo	Zn	Ni	Co	Pb	Cd	Cu
I-4	0.22	0.47	6.27	-0.84	-0.71	7.63	4.62	3.42
II-3	0.39	1.13	7.23	-0.10	-0.34	8.43	5.50	4.21
III-3	0.31	0.82	6.65	-0.74	-0.65	7.85	4.90	3.53
V-7	0.16	0.82	6.50	-0.66	-0.59	7.65	4.76	3.25
VI-11	-0.01	0.25	6.38	-0.67	-0.34	7.33	4.69	3.12
VII-12	0.17	0.36	6.07	-0.60	-0.28	6.77	4.16	2.67
VIII-8	0.06	0.13	5.66	-0.81	-0.39	6.69	3.96	2.38
Average	0.19	0.57	6.39	-0.63	-0.47	7.48	4.66	3.23



**Figure 3.** Percentages of As, Mo, Zn, Co, Cd, Cu, Ni, and Pb removed after each step of the sequential extraction procedure applied to sediments from Lake Kalimanci and adjacent areas.

phase in the observed lake sediment samples, with many also containing Zn, Pb, Cd, and As bound to this fraction.

Elements in the first 2 fractions (F1 and F2) are soluble in pure water or, in this case, the weakly acidic (due to high levels of sulfates) water of Lake Kalimanci. These highly mobile metals are therefore the most harmful to the environment. In the present study the majority of metals (Pb, Cd, Zn, Cu, and Mo) were relatively highly mobile in these 2 phases (F1 and F2), representing a serious risk to the environment and consequently also to animals and humans. Lead is the most common element in environmental pollution and can undoubtedly cause serious harm when highly bonded in the exchangeable fraction and not strongly adsorbed onto sediment particles. In such a scenario, Pb is highly available to aquatic fauna and flora. Cadmium ions can be directly desorbed into water and are known to be amongst the most mobile of metals (Kabata-Pendias & Pendias 2001). Cd is found in large amounts in the exchangeable fraction and can be easily released to the environment, eventually entering humans through the food chain. According to Campbell (1995), similarly high amounts of zinc in the exchangeable fraction represent a significant environmental risk, since Zn in this form can be easily absorbed and utilized by aquatic organisms. Copper can easily form complexes with organic matter, resulting in the widespread distribution of Cu in aquatic environments (Ikem *et al.* 2003). In this study, the metal was found dispersed equally across the Lake Kalimanci study area highly bound to organic matter, and consequently could be released under oxidizing conditions into the environment. Copper is an important nutritional element for many organisms, including humans, but too high an intake may prove toxic (Chen *et al.* 2002). Molybdenum is essential to plants at lower levels, but is toxic to animals, especially in large amounts. In the present study, Mo was the most mobile of all measured metals in the water-soluble fraction, with approximately 15% of Mo bound to this fraction at location II-3. Despite the low Mo concentrations found in surficial Lake Kalimanci sediments, such levels still pose a significant environmental risk because of the element's availability.

When the sequential extraction procedure results of the present study were compared with those of an investigation of paddy soils from nearby Kočani Plain (irrigated with water from Lake Kalimanci; Rogan *et al.* 2008), in both cases, metals such as Cd, Pb, Zn, and Cu were observed to be the most exchangeable and available for the possible contamination of the surrounding ecosystems, including via plant and animal uptake. In the majority of studies (e.g., Ebrahimpour & Mushrifah 2008;

Singh *et al.* 2008; Marcovecchio *et al.* 2010; Villalobos-Castañeda *et al.* 2010; Yan *et al.* 2010), toxic metals have mostly been found bound to the reducible and residual phase. However in this study, toxic metals including Mo, Zn, Pb, Cd, and Cu were strongly bound to the water-soluble or exchangeable phase in surficial Lake Kalimanci sediments, representing a major threat for the inhabitants of a large part of Makedonska Kamenica. It is therefore essential that toxic metal input to Lake Kalimanci, and thus also the Kočani Plain, be reduced. In order to stabilize the current condition of surficial lake sediments, complex remediation processes are required. According to Yan *et al.* (2010), sediment cleanup can only provide short-term metal reduction, with levels remaining high in both lake sediments and water. As a result, the treatment of tailings dam material and wastewater would therefore be a more effective strategy with which to restore the health of the lake and surrounding environment.

#### 4. Conclusions

The present study has reported the results of an investigation into the presence of toxic metals in Lake Kalimanci sediments. With the exception of Mo, the concentrations of remnant toxic metals such as As, Co, Cd, Cu, Ni, Pb, and Zn were generally extremely high. Increased concentrations of metals in the northern part of Lake Kalimanci are most likely related to the release of tailings dam material from mining activity at the Sasa mine. In the southern part of the lake, the observed high sediment metal content is probably associated with the presence of finer sediments (silt and clay) derived from the inflowing Kamenica River. Based on the EF classification, the magnitude of toxic metal pollution in Lake Kalimanci sediments follows the ranking of  $Cd > Pb > Zn > As > Cu > Ni > Co > Mo$ . A similar order was also determined after calculation of  $I_{geo}$  values. The results of a sequential extraction procedure revealed Pb, Cd, Zn, Cu, and Mo to be highly mobile in the water-soluble and exchangeable fractions, which undoubtedly represents an increased environmental risk. The extremely high concentrations of the studied metals and their comparatively high bioavailability could have a potentially huge impact on the surrounding ecosystem, and if no remediation or other lake environment cleaning processes are carried out in the area, the situation could also be of great concern for human health.

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